Appl. No. 09/916,017
Amdt. dated Monday, September 15, 2003
Reply to Action dated August 27, 2003

REMARKS/ARGUMENTS

Claims 1-16, 18-27 remain in the present application. Claims 1-9 have been withdrawn from consideration based on a restriction requirement. Claims 10-16, 18-27 remain under consideration. In Response to the telephonic conferences with the Examiner on August 19 and 20, 2003, and the Official Action dated August 27, 2003, please consider the following remarks.

The claims have been amended to more clearly set forth the Applicants' contribution to the art. These do not introduce new matter into the disclosure of the invention.

1. In the telephonic conference, the Examiner indicated that he has looked at our previous declaration by DeBenedetti declaration that indicates Applicants tested the ΔG for Shimogori sequence. The declaration states:

> That I have analyzed the sequence described in Shimogori et al., using a computer program called M-fold, which analyzes possible structures in RNA using Zucker's minimal energy calculations. That the only stem of possible stability is the 47 nt oligonucleotide marked as hatched boxes in the model on page 820 of the paper listed as "cgggguuuggcgggggcgcuccaugggucaggccagccgggccaccc." That this particular structure is destabilized by some bulges and G-U base pairs. That upon calculation of stability, the 5'UTR described by Shimogori would provide a secondary structure conformation having a stability ΔG of about -22 Kcal/Mol. In addition, that the construct described is only about 56% G/C-rich.

The Examiner noted that the Shimogori paper is on pages 544-548 of BBRC Vol. 223, not on page 820, which seemed to be an inconsistency. Applicants have now provided a Supplemental Declaration indicating that the plasmid called ODC-TK in the Shimogori paper is derived from a plasmid called pODC188 as described in the Kashiwagi reference (BBRC Vol. 178:815-822; 1991). This plasmid contains an mRNA open reading frame preceded by a 188 nucleotides of the 5'UTR of the ODC mRNA. The structure is the same as discussed in the previous declaration by DeBenedetti and is in fact described on page 820 of the Kashiwagi reference.

2. The Examiner then indicated that it seems like the Shimogori references teaches that the 5-prime UTR of ODC has this translational regulation. The Declaration states that that region doesn't have the proper stability -- when looking at stability in terms of ΔG. However, when looking at claim 12 (see below), the Examiner indicated that it looked like the untranslated sequence comprises 5-prime UTR of ODC of claim 12 is the same as that described by Shimogori. The Examiner felt that this could be a discrepancy between claim 10, claim 12 and the Declaration.

> Claim 12. A DNA sequence as recited in Claim 10, wherein the untranslated sequence comprises the 5' untranslated sequence selected from the group consisting of fibroblast growth factor-2, cyclin D1, proto-oncogene *c-myc*, vascular endothelial growth factor, and ornithine decarboxylase.

The particular region of ODC described by the Shimogori *et al.* reference does not provide the appropriate level of stability (ΔG ≥about 50 Kcal/Mol) to selectively regulate translation of the open reading frame and is insufficient to confer regulation by the level of eIF4E since the construct would have been translated well in the *absence* of polyamines. The full 5'UTR of the ODC mRNA has over 350 nucleotides in length and is capable of being regulated by eIF4E. Therefore, the 5'UTR of the ODC mRNA is that

portion of the mRNA that is capable of forming the proper stability in conformation and is regulatable by eIF4E.

3. Finally, the Examiner asked if we have adequately describe the stability required of the 5'UTR given that many references us a negative number to describe ΔG. The free energy "ΔG" is the free energy of an oligonucleotide, which is a measurement of an oligonucleotide duplex stability. The strength (ΔG) of the resulting complexes is measured by thermal denaturation or duplex melting. The ΔG can either be expressed as a negative or a positive number depending upon whether you are looking at the stability as a measurement of free energy stored in the structure (negative) or free energy required to melt the duplex (positive). That is, are you measuring the reaction in the **forward** (creating the folding) or **reverse** (destroying the folding) direction. Energy must be released overall to form a base-paired structure or added to pull apart the structure, and a structure's stability is determined by the amount of energy it releases or absorbs. When free energy stored in the folded structure is a negative value, then the complex formed is in the thermodynamically stable form. Predicted enthalpy, entropy and free energy of duplex formation -- the enthalpy (ΔH), entropy (ΔS), and free energy (ΔG) -- are thermodynamic state functions, related by the Gibbs equation:

$\Delta G=\Delta H-T\Delta S$ (at constant temperature and pressure)

where T is the temperature in degrees K. In practice, the enthalpy and entropy are predicted via a thermodynamic model of duplex formation and used to calculate the free energy and melting temperature.

The predicted free energy of an oligonucleotide that contains self-complementary sequences that can form intramolecular ***secondary structures*** is calculated as the most stable intramolecular structure of an oligonucleotide. "Secondary structure" as used in the present invention refers to regions of a nucleic acid sequence that, when single stranded,

have a tendency to form double-stranded hairpin structures or loops. Nucleic acids can be evaluated for their likely secondary structure by calculating the predicted ΔG of folding of each possible structure that could be formed in a particular strand of nucleic acid. Computer programs exist that can predict the secondary structure of a nucleic acid by calculating its free energy of folding. One example is the MFOLD program.

The ΔG as referred to in the specification and claims is given in absolute energy change value and is evident from the context by one skilled in the art. When expressed as a folded state free energy (a negative number), the more negative the ΔG (*i.e.*, the lower the free energy), the more stable that structure is and the more likely the formation of that double-stranded structure. The stability of a secondary structure is quantified as the amount of free energy released or used by forming base pairs or the input energy required to melt such secondary structure, which in the present case would have to be ≥ 50 Kcal/Mol, in *absolute* terms. It would be obvious to one skilled in the art that the present description describes the required energy to melt the secondary structure since a structure having a positive free energy requires work to form a configuration and hence would be unstable and could not form the required structure. Negative free energies release stored work. When quantified as the amount of free energy released or used by forming base pairs, the more negative the free energy of a structure, the more likely is formation of that structure, because more stored energy is released.

For the sake of clarity in the present invention, the stability of the oligonucleotides of the present invention has been amended to be described as "wherein the untranslated sequence further comprises a hairpin secondary structure conformation having a stability measured as folded state free energy of ΔG ≤about -50 Kcal/Mol" instead of in terms of absolute energy change. This is a more generally accepted form of notation.

Based on the foregoing amendments and remarks, as well as the attached Supplemental Declaration under 37 CFR 1.132, it is submitted that the present application

is now in form for allowance. Therefore, early reconsideration and allowance of the claims, as currently pending, are solicited.

The Assistant Commissioner for Patents is authorized to charge any deficiency or credit any overpayment to Frost Brown Todd LLC Deposit Account No. 06-2226.

Respectfully submitted,

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